Increasing cGMP-dependent protein kinase I activity attenuates cisplatin-induced kidney injury through protection of mitochondria function

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Maimaitiyiming H, Li Y, Cui W, Tong X, Norman H, Qi X, Wang S. Increasing cGMP-dependent protein kinase I activity attenuates cisplatin-induced kidney injury through protection of mitochondria function. Am J Physiol Renal Physiol 305:F881–F890, 2013. First published July 3, 2013; doi:10.1152/ajprenal.00192.2013.—Cisplatin is widely used to treat malignancies. However, its major limitation is the development of dose-dependent nephrotoxicity. The precise mechanisms of cisplatin-induced kidney damage remain unclear, and the renoprotective agents during cisplatin treatment are still lacking. Here, we demonstrated that the expression and activity of cGMP-dependent protein kinase-I (PKG-I) were reduced in cisplatin-treated renal tubular cells in vitro as well as in the kidney tissues from cisplatin-treated mice in vivo. Increasing PKG activity by both pharmacological and genetic approaches attenuated cisplatin-induced kidney cell apoptosis in vitro. This was accompanied by decreased Bax/Bcl2 ratio, caspase 3 activity, and cytochrome c release. Cisplatin-induced mitochondria membrane potential loss in the tubular cells was also prevented by increased PKG activity. All of these data suggest a protective effect of PKG on mitochondria function in renal tubular cells. Importantly, increasing PKG activity pharmacologically or genetically diminished cisplatin-induced tubular damage and preserved renal function during cisplatin treatment in vivo. Mitochondria structural and functional damage in the kidney from cisplatin-treated mice was inhibited by increased PKG activity. In addition, increasing PKG activity enhanced cisplatin-induced cell death in several cancer cell lines. Taken together, these results suggest that increasing PKG activity may be a novel option for renoprotection during cisplatin-based chemotherapy.

PKG-I: mitochondria; cisplatin; tubular cells

CISPLATIN IS ONE OF THE WIDELY used chemotherapeutic drugs. It is being used to treat a variety of solid tumors (5, 10, 14, 48). However, its therapeutic use is limited by its side effects, mostly nephrotoxicity (28, 34). Cisplatin accumulates predominantly in kidney tubular cells and induces tubular cell injury and death through multiple factors and pathways (1, 12, 31, 41, 43, 57). This dose-dependent nephrotoxicity prevents the administration of high doses of cisplatin for its chemotherapeutic efficacy. Despite intensive studies, the precise mechanisms of cisplatin-induced kidney damage remain unclear, and the renoprotective agents during cisplatin treatment are still lacking.

cGMP-dependent protein kinase (PKG) is a serine/threonine kinase consisting of a regulatory and a catalytic domain within one polypeptide chain (53). In mammalian cells, two genes encoding PKG have been identified, type I and type II (20). Type I is alternatively spliced at the first exon to encode two isoforms Iα and Iβ. These enzymes contain identical catalytic domains (3, 42). PKG-I is expressed in vascular smooth muscle cells, cardiomyocytes, endothelial cells, mesangial cells, renal tubular cells, macrophages, and other cell types (7). PKG-I has numerous effects on the vessel wall such as vasodilation, vascular permeability, induction of platelet aggregation, and regulation of smooth muscle cell proliferation (20, 30, 32, 45). In addition to regulating vessel wall function, our previous studies demonstrate a renal protective effect of PKG-I activation on diabetes or ischemia-reperfusion-induced kidney injury (29, 52). Recently, numerous studies suggest an anti-tumor effect of cGMP/PKG signaling, including induction of tumor cell apoptosis and inhibition of metastasis (13, 15, 16, 27, 47). Moreover, expression of PKG-I is reduced in many tumors compared with normal tissues (26). Based on these reports, we hypothesized that activating PKG-I pathway may prevent cisplatin-induced nephrotoxicity and enhance chemotherapeutic effect of cisplatin.

In this study, we show that PKG-I expression/activity is reduced by cisplatin treatment in proximal tubular cells in vitro as well as in the kidneys in vivo. Increasing PKG activity pharmacologically or genetically attenuates cisplatin-induced kidney cell apoptosis and tubular damage, preserving renal function during cisplatin treatment. These effects are partially through PKG-mediated mitochondria protection. In cancer cells, increasing PKG activity enhances cisplatin-induced cell death. Taken together, these results suggest that increasing PKG activity may be a novel option for renoprotection during cisplatin-based chemotherapy.

MATERIALS AND METHODS

Animals. All experiments involving mice conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Male wild-type littermates (WT) and PKG-I transgenic mice (Tg) at 8 wk of age (on C3HB6 background, generated previously by our lab) (37) were used. There were six groups: 1) Tg, saline, n = 8 mice; 2) Tg, cisplatin, n = 8 mice; 3) WT, saline, n = 8 mice; 4) WT, cisplatin, n = 8 mice; 5) WT, sildenafil, n = 6 mice; and 6) WT, sildenafil and cisplatin, n = 6 mice. Total number of transgenic mice used was 16 and total number of wild-type mice used was 28. Mice were given a single intraperitoneal injection of 30 mg/kg cisplatin and killed after 24, 48, or 72 h of cisplatin injection. Blood was collected and kidneys were harvested for various analyses. For sildenafil groups, sildenafil (dissolved in saline) was subcutaneously injected into mice (at dosage of 12 mg/kg body wt) 1 h before cisplatin injection and was given twice daily for 3 days.

Renal function, histology, immunohistochemical staining, and electron microscopy. Serum levels of creatinine were determined by using a kit from Biovision (Milpitas, CA). For histology, kidneys were fixed in formalin, embedded in paraffin, and stained with hematoxylin and
eosin. Tubular damage such as loss of brush border, tubular dilation, or cast formation was evaluated and scored (0, no damage; 1, <25%; 2, 25–50%; 3, 50–75%; 4, >75%) as previously described (39). For immunohistochemical staining, formalin-fixed and paraffin-embedded renal tissues were cut into 4- to 5-μm sections. The slides were stained with anti-active caspase 3 antibody (Cell Signaling) using VECTASTAIN Elite ABC system (Vector Lab). The color was developed with DAB. Kidney electromicroscopy (EM) was performed by using the service from the Imaging Center at University of Kentucky.

In situ enzyme staining for cytochrome c oxidase in kidney. Fresh frozen kidney tissues were cryostat sectioned at 6 μm and incubated with cytochrome c oxidase (COX) reaction solution at 37°C for 30 min to 1 h. Then slides were washed with PBS twice, covered with mounting medium, and examined under a light microscope as previously described (36, 58). The COX reaction solution consisted of 2.7 mg cytochrome c (C1345, Sigma), 15.4 mg 3,3’-diaminobenzidine tetrahydrochloride hydrate (D5545, Sigma), and 2 mg catalase (C1345, Sigma) dissolved in 10 ml of 5 mM sodium phosphate buffer. The solution was filtered after preparation and the pH was adjusted to 7.4 with 1 N NaOH.

Cell culture. 1) HK-2 cells (human renal proximal tubule cell line from American Type Culture Collection) were grown in DMEM (GIBCO, Invitrogen)/F12 containing 5.5 mM glucose with 5% FBS, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 5 ng/ml epidermal growth factor (EGF; Invitrogen). 2) Cancer cell lines including HCT-116, HT-29, MDA-MB231, and A549 were generously provided by Dr. Yadi Wu in Markey Cancer Center at University of Kentucky and cultured in DMEM media with 10% FBS. 3) Primary proximal tubular cells were isolated from wild-type littermates or PKG-I transgenic mice (male, 8–10 wk old) using the method as previously described (4). Cells were plated in collagen-coated dishes with DMEM/F12 culture medium supplemented with 25 ng/ml EGF, 1 ng/ml PGE1, 0.05 mM tri-iodothyronine, 0.05 μM hydrocortisone, 1% penicillin/streptomycin, 5% FBS and insulin-transferrin-sodium selenite medium. After overnight culture, the unattached cells were taken out and fresh media were added. These cells were cultured and treated with cisplatin (30 μM) in the presence or absence of sildenafil (10 μM) for 24 h.

Real-time quantitative PCR. Total RNA from cells and kidney tissue was extracted using RNeasy Mini Kit (Qiagen). 1) RNA was reverse transcribed to cDNA by High Capacity cDNA Reverse Transcription Kit (Invitrogen, Carlsbad, CA). Real-time quantitative PCR was performed on a MyiQ Real-time PCR Thermal Cycler (Bio-Rad) with SYBR Green PCR Master Kit (Vector Lab). The color was developed with DAB. Kidney electromicroscopy (EM) was performed by using the service from the Imaging Center at University of Kentucky.

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TUNEL staining. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay was used to analyze apoptosis in cells or kidney sections by In Situ Cell Death Detection Kit Fluorescein from Roche Applied Science (Roche Applied, Indianap-

Table 1. Sequences of primers used in the study

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward (5'-3')</th>
<th>Reverse (5'-3’)</th>
</tr>
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<tbody>
<tr>
<td>18 sRNA</td>
<td>AGTCGCCATGTTTATGTC</td>
<td>CGAACGATTTGGCAAGAT</td>
</tr>
<tr>
<td>BaX</td>
<td>ATCGAAACTCATGGCTGGACA</td>
<td>AGCCCCATTTTCTGCAGTGTGA</td>
</tr>
<tr>
<td>Bel-2</td>
<td>AAACGCATGTTCATCGACAGA</td>
<td>CACATGCGCAGCATTAA</td>
</tr>
<tr>
<td>PGCG-1e</td>
<td>TGGTCAGCGATGTTACTTCTAGC</td>
<td>AGATGACGAGCATTTGGGTA</td>
</tr>
<tr>
<td>28 sRNA</td>
<td>TCCAAGTTTGGTAACATCAA</td>
<td>TGTATGGGTTACTCCGCTTGA</td>
</tr>
<tr>
<td>ND1</td>
<td>TCCAAGTTTGGTAACATCAA</td>
<td>TGTATGGGTTACTCCGCTTGA</td>
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Fig. 1. Downregulation of cGMP-dependent protein kinase-I (PKG-I) expression and activity in the kidney from cisplatin-treated mice and HK-2 cells. A: Wild-type mice were injected with cisplatin (30 mg/kg). After 3 days, kidneys were harvested. PKG protein levels (A) and PKG activity reflected by p-vasodilator-stimulated phosphoprotein (VASP) at serine 239 (B) in the kidney homogenates were determined by immunoblotting. C: HK-2 cells were treated with 30 μM cisplatin for 24 h. PKG-I protein levels and p-VASP at serine 239 levels in the cell lysates were determined by immunoblotting. Results are means ± SE (n = 6 mice/group for A and B or n = 3 separate experiments for C). **P < 0.01 vs. saline, #P < 0.05 vs. control.
olise, IN) and performed according to the protocol manual. After being stained, mounting media containing DAPI were applied onto slides. The slides were examined by fluorescence microscopy and images were taken. Percent of apoptosis for each slide was calculated by counting TUNEL-positive nuclei under high magnification (×40) and normalizing to total number of nuclei in one field. Total of five to eight fields for each slide were examined.

Caspase 3 activity assay. Activity of caspase-3 in cell extracts was detected using a caspase-3 calorimetric assay kit (R&D Systems) based on cleavage of the peptide by the caspase to release the chromophore p-nitroanilide, which can be measured at a wavelength of 405 nm. Preparation of cell extracts and analysis of caspase-3 activity were performed according to the manufacturer’s protocol.

Measurement of the mitochondria membrane potential in cultured cells. Mitochondria membrane potential was examined using JC-1 mitochondria potential sensor (Invitrogen) based on that potential-dependent accumulation of JC-1 causes the fluorescence shift from green to red. HK-2 cells and primary proximal tubule cells were cultured and treated with cisplatin (30 μM) in the presence or absence of sildenafil (10 μM) for 24 h. After treatment, cells were incubated with JC-1 dye (1 μg/ml) at 37°C for 30 min. Fluorescence intensity was measured using fluorescence microplate reader. The mitochondrial membrane potential was calculated as the ratio of red to green fluorescence.

Statistical analysis. All data are expressed as means ± SE. ANOVA was used to analyze variation within the group. Student’s t-tests were used to compare variation between groups. Statistical significance will be accepted at a value of P < 0.05.

RESULTS

PKG-I protein levels as well as its activity are decreased in cisplatin-treated renal proximal tubular cells and mouse kidneys. To analyze PKG-I protein levels/activity changes in cisplatin-induced nephrotoxicity, we used a well-established mouse model, a single-dose injection of cisplatin-induced acute kidney injury (17). After 72 h of cisplatin injection (30 mg/kg body wt), mice were killed and kidneys were harvested. Protein levels of PKG-I in the kidney were determined by immunoblotting. PKG activity in the kidney was analyzed by phosphorylation of vasodilator-
stimulated phosphoprotein (VASP) at serine 239. VASP is a ubiquitously expressed endogenous substrate for PKG, and phosphorylation of VASP at serine 239 has been used as a biomarker for PKG activation (49). As shown in Fig. 1, A and B, PKG-I protein levels as well as PKG activity were significantly reduced in the kidney from cisplatin-treated mice. In cultured proximal tubular cells (HK-2), cisplatin also reduced PKG-I protein levels and its activity (Fig. 1C). Together, these in vivo and in vitro analyses demonstrate the decreased PKG activity in cisplatin-induced nephrotoxicity.

**Increasing PKG activity attenuates cisplatin-induced apoptosis in renal tubular cells.** To determine the effect of increasing PKG activity on cisplatin-induced renal tubular cell apoptosis, both pharmacological and genetic approaches were used to increase PKG activity in renal tubular cells. First, proximal tubular cells (HK-2 cells) were cultured and treated with sildenafil (a PDE5 inhibitor) to increase PKG activity before cisplatin treatment. The effect of increased PKG activity on cisplatin-induced apoptosis was then investigated. As shown in Fig. 2A, cisplatin-induced HK-2 cell apoptosis (by TUNEL staining) was significantly attenuated by sildenafil treatment. Furthermore, sildenafil treatment inhibited the critical mitochondria events of apoptosis such as increased caspase 3 activity and cytochrome c release by cisplatin treatment (Fig. 2B and C). We also showed that sildenafil treatment prevented cisplatin-induced mitochondria membrane potential loss (detected by a JC-1 mitochondria potential sensor assay; Fig. 2D).

In addition to using the above pharmacological approach to increase PKG activity, we isolated primary proximal tubular cells from PKG-I transgenic mice generated by our laboratory previously (37). The Western blotting demonstrated that PKG transgene was overexpressed in these cells (Fig. 3A), accompanied by increased PKG activity (Fig. 3B). Cisplatin treatment induced mitochondria injury in proximal tubular cells isolated from wild-type mice, including cytochrome c release, increased Bax/Bcl2 ratio, Bax translocation into mitochondria, and increased and decreased mitochondria membrane potential (JC-1 assay). These cisplatin-induced damages were attenuated or prevented in proximal tubular cells isolated from PKG transgenic mice (Fig. 3). Taken together, these data demonstrate that increasing PKG activity attenuates cisplatin-induced renal tubular cell apoptosis partially through mitochondria protection.

**Increasing PKG activity attenuates cisplatin-induced nephrotoxicity in mice.** We determined whether increasing PKG activity protects mice from cisplatin-induced acute kidney injury in vivo in a mouse model. PKG transgenic mice and wild-type mice with sildenafil treatment to increase PKG activity were used. As shown in Fig. 4A, cisplatin induced acute renal injury in wild-type mice in 3 days, increasing serum creatinine levels. The creatinine increases were significantly decreased by sildenafil treatment in wild-type mice or in PKG transgenic mice. Consistently, increasing PKG activity suppressed cisplatin-induced renal tubular damage (Fig. 4, B and D) and apoptosis (Fig. 5A). Cisplatin-induced increase in caspase 3 activity and cytochrome c release by cisplatin treatment (Fig. 2C).

**Fig. 3.** Proximal tubular cells isolated from PKG-I transgenic mice protected mitochondria function during cisplatin treatment. Primary proximal tubular cells were isolated from wild-type and PKG-I transgenic mice. A: transgene (the catalytic domain of PKG-I) expression in the cell lysates was determined by immunoblotting. B: PKG activity in the cell lysate was determined by p-VASP at serine 239 levels by immunoblotting. C: cells were treated with 30 μM cisplatin for 24 h. Released cytochrome c into cytosolic fraction separated from mitochondria fraction was detected by immunoblotting. D: mRNA levels of Bax/Bcl2 ratio were determined by real-time PCR. E: Bax translocation into mitochondria was determined by immunoblotting. F: loss of mitochondria potential was determined by JC-1 mitochondria potential sensor assay. The results are expressed as means ± SE (n = 4 mice/group). WT, wild-type; Tg, PKG-I transgenic mice. *P < 0.05 vs. WT-control. **P < 0.01 vs. WT-control. #P < 0.05 vs. WT + Cis.
release in the kidney were inhibited by sildenafil treatment or PKG overexpression (Fig. 5, B, C, D). Together, these results indicate that increasing PKG activity attenuates cisplatin-induced nephrotoxicity in mice.

Increasing PKG activity prevents cisplatin-induced kidney mitochondria damage. The above studies suggest that increasing PKG activity protects mitochondria from cisplatin injury. We further determined the effect of increased PKG activity on kidney mitochondria mass and morphological and functional changes in cisplatin-injected mice. As shown in Fig. 6A, in wild-type mice, cisplatin treatment led to decreased mitochondria DNA copy number in renal tissues. The decrease was detected at day 1 of cisplatin treatment, a time point before kidney injury. In contrast, in PKG transgenic mice, cisplatin treatment did not reduce kidney mitochondria DNA copy number. Since increasing PKG activity has been shown to stimulate mitochondria biogenesis (21, 35, 38), we determined the expression of PGC-1α levels (a master regulator of mitochondrial biogenesis) in the kidney. As shown in Fig. 6B, cisplatin treatment reduced expression of PGC-1α in the kidney from wild-type mice, but not from PKG-I transgenic mice. EM revealed the swollen mitochondria with disruption of cristae in the kidney from cisplatin-treated wild-type mice. This mitochondria structural damage was attenuated by overexpression of PKG (Fig. 6C). In addition, we examined COX activity in the kidney using in situ enzyme chemistry approach. COX is a mitochondria electron transport chain enzyme. Consistent with previous reports (36, 58), we found that cisplatin treatment reduced COX enzyme activity in kidney tubular epithelium, which was inhibited by PKG overexpression (Fig. 6, D and E). Together, these results suggest that increasing PKG activity prevents cisplatin-induced mitochondria morphological and functional damages.

Increasing PKG activity enhances cisplatin-induced cancer cell apoptosis. We examined the effects of sildenafil treatment on cisplatin-induced apoptosis in multiple cancer cell lines in vitro. Cancer cells were pretreated with sildenafil for 1 h before addition of cisplatin. Figure 7 showed that sildenafil pretreatment markedly increased apoptosis during cisplatin treatment in three cancer cell lines tested.

**DISCUSSION**

In this study, we first demonstrated that decreased PKG-I signaling is an important regulator of cisplatin-induced nephrotoxicity. Importantly, increasing PKG activity by both pharmaco-
logical and genetic approaches attenuated cisplatin-induced kidney cell apoptosis and tubular damage, preserving renal function during cisplatin treatment. Such protection was likely due to an ability of PKG to protect mitochondria. In cancer cells, increasing PKG activity preserves or even enhances cisplatin-induced cell death. Taken together, these results suggest that increasing PKG activity may be a novel approach for renoprotection during cisplatin-based chemotherapy.

PKG is a downstream signaling molecule of nitric oxide and cGMP. It is a serine/threonine kinase, consisting of a regulatory and a catalytic domain. Binding of cGMP by the regulatory domain leads to activation of the catalytic domain and...
Fig. 6. Increasing PKG activity prevented cisplatin-induced mitochondria damage in the kidney from mice in vivo. A: male wild-type or PKG-I transgenic mice were injected with cisplatin (30 mg/kg) before collection of kidneys at days 0–3. Mitochondria DNA copy number in the kidney was determined by real-time PCR. B: mRNA and levels of PGC-1α in the kidneys from cisplatin-treated mice were determined by real-time PCR and immunoblotting, respectively. C: kidney tubular cell mitochondria structure after 3 days of cisplatin injection was demonstrated by electronmicroscopy. Original magnification ×4,800. D: staining (brown) for cytochrome c oxidase enzyme activity on sections of fresh frozen kidneys 3 days after cisplatin treatment. Representative images were shown. The positive area was calculated (E). Original magnification ×200. Data shown are means ± SE (n = 3 mice/group). *P < 0.05. **P < 0.01. *P < 0.05 vs. WT 24-h Cis. ##P < 0.01 vs. WT 48-h Cis. &P < 0.05 vs. 72-h Cis.
PKG-I in cisplatin-induced nephrotoxicity

increases PKG activity (53). With the identification of decreased PKG activity in cisplatin-treated proximal tubular cells or in the kidneys from cisplatin-treated mice, in this study, we tested whether increasing PKG activity could prevent cisplatin-induced nephrotoxicity. Both genetic and pharmacological approaches were utilized to increase PKG activity in our studies. For the genetic approach, we used the transgenic mice with overexpression of the catalytic domain of PKG-I (constitutively active PKG-I) in the kidney generated by our lab previously (29). For the pharmacological approach, a phosphodiesterase type 5 (PDE5) inhibitor (sildenafil, specifically in vivo) was usedwareable drug, which is widely used in clinics to treat erectile dysfunction and pulmonary hypertension (23, 33). In this study, we found that increasing PKG activity by the above two approaches attenuated cisplatin-induced acute kidney injury, suggesting that we not only reveal a new signaling pathway in cisplatin-induced nephrotoxicity but also provide a new renoprotection strategy. Importantly, the renoprotective effect of sildenafil on cisplatin treatment has clinical significance.

Cisplatin-induced renal cell death involves multiple factors and pathways, and mitochondria damage is one of the important mechanisms (4, 34, 36, 41, 43, 56, 58). Cisplatin-induced mitochondria damage has been observed in cell culture or animal models by numerous studies. In agreement with previous reports (4, 36, 51, 58), we found that cisplatin treatment significantly reduced mitochondria mass and induced mitochondria morphological and functional damages such as mitochondrial membrane potential loss, released cytochrome c from mitochondria into cytosolic fraction, and reduced mitochondria COX activity. All of these cisplatin-induced mitochondria injuries were attenuated or prevented by increased PKG activity. One mechanism of this PKG-mediated mitochondria protection may be the stimulatory effect of PKG on mitochondria biogenesis by regulating PGC1-α expression (21, 35, 38). PGC1-α is a master regulator for mitochondria biogenesis (54). Consistently, we found that cisplatin treatment reduced PGC-1α expression in the kidney from wild-type mice, but not from PKG-I transgenic mice (Fig. 6B). In addition, activation of PKG has been shown to protect heart mitochondria by inhibiting the opening of mitochondria permeability transition pore via opening mitochondria K<sub>ATP</sub> channel (8, 9). Whether this also occurred in the cisplatin model in the kidney is unknown and warrants further investigation.

PKG-I is a multifunctional protein. Previous studies suggested that PKG may be proapoptotic or anti-apoptotic, depending on cell types (19, 50). Recently, a proapoptotic role of PKG has been shown in various cancer cell types, linking cGMP/PKG signaling to anti-tumor activities (13, 15, 16, 27, 47). Expression of PKG-I is downregulated in multiple tumor tissues compared with respective normal tissues (26). Activation of cGMP/PKG pathway inhibits breast or colon cancer cell growth and angiogenesis, and it induces these cells’ apoptosis (13, 27, 47). A death-associated protein kinase 2 has been identified to be the substrate of PKG-I and mediates PKG-I-induced breast cancer cell apoptosis (25). Moreover, studies showed that sildenafil may enhance the sensitivity of certain types of cancer to chemotherapeutic drugs (2, 11, 46). Consistently, in this study, sildenafil treatment to activate cGMP/PKG preserved or even enhanced cisplatin-induced apoptosis in several cancer cell lines. In contrast to the proapoptotic effect of cGMP/PKG on cancer cells, our studies demonstrated an anti-apoptotic effect of activation of cGMP/PKG on cisplatin-induced apoptosis in renal proximal tubular cells. This may be due to a cell type-specific effect or a difference in cisplatin uptake between renal tubular cells and cancer cells. Recent studies have identified two types of membrane transporter that mediate cisplatin transporting into cells: copper transporter 1 (Ctr1) and organic cation transporter 2 (OCT2). Ctr1 mediates cisplatin transporting into mammalian cells including ovarian cancer cells and kidney cells in vitro (22, 24, 40). In addition, OCT2 has been shown to be a critical transporter for cisplatin uptake in proximal tubules in both animals and humans (55). It also mediates cisplatin-induced renal toxicity (6, 18). OCT2 in kidney cells can be inhibited by cGMP (44). However, in some cisplatin-sensitive tumor cell lines, OCT2 expression was not found (6). Together, these reports lead us to speculate that activation of cGMP/PKG pathway may inhibit OCT2-mediated cisplatin uptake in renal proximal tubular cells (but not in tumor cells) and then protect these renal cells from cisplatin-induced cytoxicity. This mechanism needs to be further investigated in the future.

In summary, our studies first identified the decreased PKG-I expression/activity in renal tubular cells during cisplatin treatment. Increasing PKG activity genetically or pharmacologically prevents cisplatin-induced kidney cell apoptosis and tubular damage partially through mitochondria protection. Importantly, increasing PKG activity preserves or even enhances the anticancer effect of cisplatin in several cancer cell lines, suggesting that pharmacologically increasing PKG activity (using clinically available drug sildenafil) may serve as an effective strategy to protect the kidney during cisplatin-based chemotherapy.

GRANTS

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Fig. 7. Effect of sildenafil treatment on cisplatin-induced apoptosis in several cancer cell lines. Indicated cancer cell lines were pretreated with sildenafil (10 μM) for 1 h and then treated with cisplatin (30 μM) for 24 h. Apoptosis was determined as described in MATERIALS AND METHODS. The results are expressed as means ± SE (n = 5 separate experiments). *P < 0.05 vs. cisplatin. **P < 0.01 vs. cisplatin.
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